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13. ABSTRACT (Maximum 200 words) The major objective of the grant was to develop biomarkers to establish exposures and risks at Air Force sites, and to investigate chemicals peculiarly associated with them. The accomplishments include a number of analytical techniques and biomarker studies that can be applied when the situations demand. Pesticide studies were undertaken only when the results were important for development of methods suitable for use at Air Force sites involving chemicals known to occur there.			
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The major objective of the grant was to develop biomarkers to establish exposures and risks at Air Force sites, and to investigate chemicals peculiarly associated with them. The accomplishments include a number of analytical techniques and biomarker studies that can be applied when the situations demand. Pesticide studies were undertaken only when the results were important for development of methods suitable for use at Air Force sites involving chemicals known to occur there. A brief summary of accomplishments follows. More detailed discussions are in the annual reports.

**Biomarkers:*****Cholinesterases***

Organophosphate pesticides and chemical warfare agents are often monitored by cholinesterase measurements. Major improvements have been made in the clinical and field methods used to determine blood enzymes in man, laboratory animals and wildlife.

***Neuropathy Target Esterase***

Air Force brake fluids once contained the ortho isomer of tri-cresyl phosphate. A substance associated with outbreaks of a long term neuropathy. Purification methods were studied for neuropathy target esterase (an enzyme associated with the organophosphate induced delayed neuropathy) and we made a good beginning on methodology to establish its localization in the nervous system.

***Fecal Testosterone in Mice***

Wild animals are important sentinels at Air Force sites and other ecosystems. A method to determine the testosterone level in feces of mice was developed and subjected to a field test at Mare Island. The method will permit non-invasive determination of testosterone levels in wild animals allowing us to establish their reproductive state. Such studies may provide early warning signs of environmental problems, since reproduction is a sensitive feature of the physiology of animals.

***Cell and Organ Cultures***

Alternatives to animal testing were studied using metals and organophosphates, TCDD-like molecules and nerve, muscle and liver cultures and liver from bird embryos. Highly differentiated surface cultures and brain cell reaggregates were developed and studied. Induction of EROD activity could be demonstrated in embryos and liver cells from wild birds and interactions of cadmium and organophosphates were demonstrated in vitro with muscle and nerve cells.

**Analytical**

The analytical part of the project focused on improving methods of extraction of environmental contaminants important to the Air Force using techniques such as super critical extractions. Soils were analyzed for important contaminants from Air Force sites.

## **Conclusion**

The project ended with the scientists poised to carry out combined laboratory and field studies with biomarker and analytical techniques at selected Air Force sites such as Beale Air Force base.

## **References**

David, M D; Seiber, J N.

Presence of organophosphorus hydraulic fluid components in soils at U.S. Air Force bases. (213th National Meeting of the American Chemical Society, San Francisco, California, USA, April 13-17, 1997. )

Woodrow, J E; Seiber, J N; Baker, L W.

Correlation techniques for estimating pesticide volatilization flux and downwind concentrations.

Environmental Science & Technology, v.31, n.2, (1997): 523-529.

Wujcik, C E; Seiber, J N.

Supercritical fluid extraction of 2,4,6-trinitrotoluene and 1,3,5-trinitrobenzene from soil.

Journal of Environmental Science and Health Part A Environmental Science and Engineering & Toxic and Hazardous Substance Control, v.31, n.6, (1996): 1361-1377.

David, M D; Seiber, J N.

Comparison of soil extraction techniques for organophosphorus hydraulic fluids. (211th American Chemical Society National Meeting, New Orleans, Louisiana, USA, March 24-28, 1996. )

Wolfe, M F; Hinton, D E; Seiber, J N.

Aqueous sample preparation for bioassay using supercritical fluid extraction.

Environmental Toxicology and Chemistry, v.14, n.6, (1995): 1001-1009.

David, M D; Seiber, J N.

Accelerated hydrolysis of organophosphorus hydraulic fluids and related organophosphates using sodium-perborate generated HO-2. (209th American Chemical Society National Meeting, Anaheim, California, USA, April...)

### **7. (BIOSIS result)**

Wilson, B W; Billitti, J E; David, M; Lasley, B L; Fry, D M; Seiber, J N.

Impact of organophosphates on ecosystems. (209th American Chemical Society National Meeting, Anaheim, California, USA, April 2-6, 1995. )

Henderson, J D; Yamamoto, J T; Fry, D M; Seiber, J N; Wilson, B W.  
Oral and dermal toxicity of organophosphate pesticides in the domestic  
pigeon (*Columba livia*).  
*Bulletin of Environmental Contamination and Toxicology*, v.52, n.5,  
(1994): 633-640.

Wolfe, M F; Seiber, J N; Hinton, D E.  
Aqueous Sample Preparation for Bioassay Using Supercritical Fluid  
Extraction.

Davis, J A; Fry, D M; Wilson, B W.  
Hepatic ethoxyresorufin-O-deethylase activity and inducibility in wild  
populations of double-crested cormorants (*Phalacrocorax auritus*).  
*Environmental Toxicology and Chemistry*, v.16, n.7, (1997): 1441-1449.

Coatney, E M; Wilson, B W.  
Cadmium depression of acetylcholinesterase in primary chicken brain  
culture. (Annual Meeting of the 6th International Congress on Cell Biology  
and the 36th American Society for Cell Biology, San Francisco,...  
*Molecular Biology of the Cell*, v.7, n.SUPPL., (1996): 651A.

Wilson, B W; Sanborn, J R; O'Malley, M A; Henderson, J D; Billitti, J R.  
Monitoring the pesticide-exposed worker.  
*Occupational Medicine (Philadelphia)*, v.12, n.2, (1997): 347-363.

Wilson, B W; Padilla, S; Henderson, J D; Brimijoin, S; Dass, P D; Elliot,  
G; Jarger, B; Lanz, D; Pearson, R; Spies, R.  
Factors in standardizing automated cholinesterase assays.  
*Journal of Toxicology and Environmental Health*, v.48, n.2, (1996):  
187-195.

Mackay, C E; Hammock, B D; Wilson, B W.  
Identification and isolation of a 155-kDa protein with neuropathy target  
esterase activity.  
*Fundamental and Applied Toxicology*, v.30, n.1, (1996): 23-30.

Borhan, B; Hammock, B; Seifert, J; Wilson, B W.  
Methyl and phenyl esters and thioesters of carboxylic acids as surrogate  
substrates for microassay of proteinase K esterase activity.  
*Fresenius' Journal of Analytical Chemistry*, v.354, n.4, (1996): 490-492.

Bartkowiak, D J; Wilson, B W.  
Avian plasma carboxylesterase activity as a potential biomarker of  
organophosphate pesticide exposure.  
*Environmental Toxicology and Chemistry*, v.14, n.12, (1995): 2149-2153.

Borhan, B; Ko, Y; Mackay, C; Wilson, B W; Kurth, M J; Hammock, B D.  
Development of surrogate substrates for neuropathy target esterase.  
*Biochimica et Biophysica Acta*, v.1250, n.2, (1995): 171-182.

Wilson, B W; Billitti, J E; David, M; Lasley, B L; Fry, D M; Seiber, J N.  
Impact of organophosphates on ecosystems. (209th American Chemical  
Society National Meeting, Anaheim, California, USA, April 2-6, 1995. )  
Abstracts of Papers American Chemical Society, v.209, n.1-2, (1995): AGRO  
119.

Billitti, J E; Henderson, J D; Sanborn, J R; Padilla, S; Wilson, B W.  
Standardization of DTNB colorimetric cholinesterase measurements for  
human monitoring and animal investigations. (209th American Chemical  
Society National Meeting, Anaheim, California, USA, April 2-6, 1995. )  
Abstracts of Papers American Chemical Society, v.209, n.1-2, (1995): AGRO  
107.

Davis, J A; Fry, D M; Wilson, B W.  
Fluorescence-based catalytic assays for measuring cytochrome P-450  
induction in birds. (209th American Chemical Society National Meeting,  
Anaheim, California, USA, April 2-6, 1995. )  
Abstracts of Papers American Chemical Society, v.209, n.1-2, (1995): AGRO 105.

Seifert, J; Wilson, B W.  
Solubilization of neuropathy target esterase and other phenyl valerate  
carboxylesterases from chicken embryonic brain by phospholipase A-2.  
Comparative Biochemistry and Physiology C Pharmacology Toxicology and  
Endocrinology, v.108, n.3, (1994): 337-341.

Funk, K A; Henderson, J D; Liu, C-H; Higgins, R J; Wilson, B W.  
Neuropathology of organophosphate-induced delayed neuropathy (OPIDN) in  
young chicks.  
Archives of Toxicology, v.68, n.5, (1994): 308-316.

Henderson, J D; Yamamoto, J T; Fry, D M; Seiber, J N; Wilson, B W.  
Oral and dermal toxicity of organophosphate pesticides in the domestic  
pigeon (*Columba livia*).  
Bulletin of Environmental Contamination and Toxicology, v.52, n.5,  
(1994): 633-640.

Funk, K A; Liu, C-H; Wilson, B W; Higgins, R J.  
Avian Embryonic Brain Reaggregate Culture System: I. Characterization for  
Organophosphorus Compound Toxicity Studies.  
Toxicology and Applied Pharmacology, v.124, n.1, (1994): 149-158.

Funk, K A; Liu, C-H; Higgins, R J; Wilson, B W.  
Avian Embryonic Brain Reaggregate Culture System: II. NTE Activity Discriminates between Effects of a Single Neuropathic or Nonneuropathic Organophosphorus Compound Exposure.  
Toxicology and Applied Pharmacology, v.124, n.1, (1994): 159-163.

Liu, C H; Higgins, R J; Buster, D; Sanborn, J R; Wilson, B W.  
The effect of organophosphates on a chicken brain or sea urchin egg kinesin-driven microtubule motility assay.  
Toxicology Letters (Amsterdam), v.69, n.3, (1993): 239-247.

Thomas, T C; Szekacs, A; Hammock, B D; Wilson, B W; Mcnamee, M G.  
Affinity Chromatography of Neuropathy Target Esterase.  
Chemico-Biological Interactions, v.87, n.1-3, (1993): 347-360.

Mackay, C; Seifert, J; Wilson, B W.  
Solubilization of Brain Neuropathy Target Esterase Nte by Phospholipase A-2 Pl A-2.  
Abstracts of Papers American Chemical Society, v.206, n.1-2, (1993): AGRO 66.

Henderson, J D; Sanborn, J R; Nieberg, P A; Schneider, F A; O'malley, M A; Hernandez, B Z; Smith, C R; Wilson, B W.  
Comparison of Test-Mate Op Kit Optical Plate Reader and Clinical Laboratory Assays for Cholinesterases.

Stein, R W; Yamamoto, J T; Fry, D M; Ottum, N D; Wilson, B W; Sieber, J N; McChesney, M M; Richardson, E E; Joseph, V J.  
Comparative Evaluations of Hematologic Parameters of Red-Tailed Hawks and American Kestrels Trapped in California.  
Journal of Raptor Research, v.27, n.1, (1993): 93.

Ottum, N D; Fry, D M; Wilson, B W; Yamamoto, J T; Sieber, J N; Mcchesney, M M.  
Home Range Distribution and Habitat Usage of Red-Tailed Hawks and Red-Shouldered Hawks During Dormant Spray Season in the Central Valley.  
Journal of Raptor Research, v.27, n.1, (1993): 78.

Fry, D M; Wilson, B W; Ottum, N D; Yamamoto, J T; Stein, R W; Sieber, J N; McChesney, M M; Richardson, E.  
Analysis of Pesticide Exposure Risk To Red-Tailed Hawks Wintering in Almond Orchards in California.  
Journal of Raptor Research, v.27, n.1, (1993): 69-70.